Transcript

Slide 1: Cardiomyopathy = heart muscle disease

Blood enters the left atrium and then goes on to the left ventricle. It's the left side of the heart that we are particularly interested in with most of the cardiomyopathies. The left ventricle ejects blood out into the aorta and off to the body. Hypertrophic cardiomyopathy particularly affects the left ventricle.

Slide 2:

Hypertrophic cardiomyopathy (HCM) is the most common of all the cardiomyopathies in cats and is characterised by increased left ventricular wall thickness. There is some overlap with the other types of cardiomyopathy. Restrictive cardiomyopathy (RCM) may have similar features to hypertrophic cardiomyopathy, where the left atrium can be dilated but generally the left ventricle is fairly normal. The other cardiomyopathy that has some overlap with hypertrophic cardiomyopathy is dilated cardiomyopathy (DCM) and in this form of heart muscle disease the heart doesn't contract very well. A fourth type of cardiomyopathy that is relatively unrelated is called arrhythmogenic right ventricular cardiomyopathy (ARVC) and this, as the name suggests, mostly affects the right ventricle although it can affect the left ventricle as well.

Slide 3:

If we look specifically at the left heart, blood returns from the lungs into the pulmonary veins, into the left atrium and is then ejected into the left ventricle before being pumped out into the aorta and off to the body.

With hypertrophic cardiomyopathy (HCM), the key difference is the thickening of the walls of the left ventricle (LV). As the disease becomes more severe, we may start to see enlargement of the left atrium and at that stage the cat starts to be at risk of developing congestive heart failure or a clot.

Restrictive cardiomyopathy (RCM) differs in that wall thickness is normal and the left ventricle can look relatively normal, but in fact it is stiffer than normal so the left ventricle still doesn't fill very well and generally there will be an enlarged left atrium. These are the characteristics of the most common types of cardiomyopathy that we see in Norwegian Forest Cats (NFCs).

Slide 4:

We generally assess the shape and function of the heart with echocardiography. Here we have an echo of a normal cat's heart, we have the left atrium here, the left ventricle here and these are the walls of the heart (left ventricle) and the aorta is here; corresponding with the image that we have above.

Here is a cat with hypertrophic cardiomyopathy. You can see immediately that the walls of the heart (left ventricle) are much thicker than a normal heart. Here the left atrium is a relatively normal size and this is the aorta, again corresponding with our diagram.

In restrictive cardiomyopathy, you can see the walls of the left ventricle are relatively normal thickness but we have a very big left atrium here and a very big right atrium is visible as well. This is one way in which echocardiography, cardiac ultrasound, can be used to demonstrate the shape and function of the different heart chambers.

Slide 5: Why do we care about cardiomyopathy?

One of the things we fear with hypertrophic cardiomyopathy is that they can develop congestive heart failure and this can lead to fluid in the lungs. This can happen because having the very stiff, thickened walls of the left ventricle means that the ventricle doesn't fill very easily. This can lead to a build-up of pressure in the left atrium, so the blood draining from the lungs into the pulmonary veins and into the left atrium can't drain very easily and the pressure goes up in the vessels in the lungs. This can lead to fluid build-up in the lung tissue and cats will become breathless and may end up dying of poor oxygenation due to the fluid in their lungs; this is a life-threatening condition.

Slide 6:

Another catastrophic complication of cardiomyopathy is when a clot develops in the heart. As the ventricle is stiff and doesn't fill with blood very easily, the left atrium becomes enlarged. Blood swirls around in the enlarged left atrium and doesn't move into the left ventricle properly, so you can start to get a clot forming within the heart. If this clot then moves into the left ventricle and out into the circulation, it can block the blood supply to the major arteries and all too often we see cats presenting with sudden paralysis associated with an obstruction of blood flow to the back legs. This is one of the worst possible things that can happen to a cat that develops cardiomyopathy.

Slide 7:

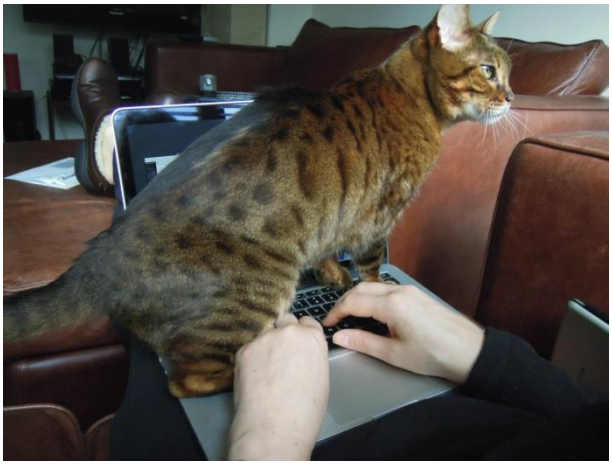
There are cats that we just lose suddenly, sometimes with no warning at all. This may be associated with abnormal excitability of the heart muscle cells so that they become irritable and start depolarising (electrical activity) spontaneously; this can lead to fatal heart rhythms. This is another important consequence of cardiomyopathy and we are only just starting to learn about some of the risk factors for this happening. I think this is a consequence of cardiomyopathy that is underreported, because many people may not even know their cat had cardiomyopathy and the first sign of problems may be when they are just found dead.

Slide 8: How did I get to become involved in hypertrophic cardiomyopathy?

I was interested in heart disease in cats ever since I was a veterinary student. This increased further and became more personal when I took on a young Bengal cat when I was working in the States who had hypertrophic cardiomyopathy. The breeders didn't want her if she couldn't be used for breeding and I found myself taking on this 6 month old Bengal. I thought I am not going to be in the States forever, but she will probably die in a year or so; it shouldn't be a problem when I move back to the UK.

Slide 9:

Fifteen years later, this was me trying to put this webinar together yesterday and she was trying to make my life difficult as usual. She has still not shown any signs of heart failure, she is still living with her hypertrophic cardiomyopathy. She has made me realise how little we knew about prognosis in cats with HCM and is one of the reasons I have wanted to do further research in hypertrophic cardiomyopathy.



Slide 10:

The other thing was that we knew many cats in my daily work I would see with signs of heart failure associated with cardiomyopathy. As cardiologists we see some of the worst consequences, but some of the work we have done has made us realise that probably the majority of cats with hypertrophic cardiomyopathy actually live normal lives like my Bengal. It's a very variable disease and some cats will have life-threatening consequences and will die of their cardiomyopathy and many other cats will never develop clinical signs and will have a completely normal lifespan.

- **Participant**: Is your Bengal on any type of medication?
- **Virginia**: No, she's not on anything

Slide 11: Why Norwegian Forest cat cardiomyopathy?

The reason has to be Yve Hamilton-Bruce! It's thanks to her contacting us asking if anybody would look further into cardiomyopathy in NFCs that we became hooked. There were some characteristics of NFC cardiomyopathy that were different to cardiomyopathy in some other breeds and Yve was kind enough to push us into looking into this disease in NFCs further as well as put us in touch with owners of a number of cats who were thought to be affected. It's thanks to Yve that we started going down this path.

Slide 12:

Norwegian Forest Cat cardiomyopathy is an interesting type of cardiomyopathy and I think we still have lots to learn. One of the things that struck me was that there were multiple instances of really relatively young cats, often under 2 years of age, that were developing the really serious consequences of cardiomyopathy. Although there may be NFCs who are diagnosed with cardiomyopathy, the fact that we were seeing young cats being severely affected was certainly something that caught our interest. Some of the features both on echocardiography and on post-

mortem (necropsy) were that some cats have features of hypertrophic cardiomyopathy and others had features more characteristic of restrictive cardiomyopathy; that in itself is very interesting. Restrictive cardiomyopathy is a relatively uncommon condition.

Slide 13:

Thanks to Yve we started gathering more information not just doing screening with echocardiography, but looking at cats that had died and finding changes gain that had some features of hypertrophic cardiomyopathy and some features of restrictive cardiomyopathy. It is devastating to lose any cat with cardiomyopathy, but so much worse when you lose them in their youth.

Slide 14:

I also have to thank Imke Maerz, who was a resident at the time we first started looking at NFCs. Imke took on a lot of this screening of cats that were related to those known to have been affected with cardiomyopathy or known to have died of cardiomyopathy.

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There are some interesting findings just on physical examination. We expect in normal healthy cats a large proportion to have heart murmurs, and not all cats with heart murmurs will have cardiomyopathy. What struck us when examining NFCs was how uncommon it was to hear a heart murmur. Heart murmurs are caused by turbulent blood flow as it goes through the heart and we don't necessarily hear this even in NFCs that are affected with cardiomyopathy.

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Imke did a lot of this work and we tried to screen as many cats as possible that were related to cats known to have been affected with cardiomyopathy. Although we weren't able to screen every related cat, we found that a large proportion of these cats were diagnosed with cardiomyopathy; or at least were in that grey zone (equivocal) where we couldn't clearly call them normal but we couldn't call them affected with cardiomyopathy. Cats that were equivocal are shown in grey, cats that were affected are shown in black, cats that were normal are shown with a dark outline and clear centre (see diagram). This looks strongly suggestive of an autosomal dominant pattern of inheritance. This strongly suggested we were dealing with a familiar or genetic problem.

Slide 17: Problems in NFC Cardiomyopathy

This is a difficult form of cardiomyopathy to be dealing with, I'm afraid you have chosen a breed that has some big problems with investigating this type of cardiomyopathy.

The first is that we don't have to see dramatic thickening of the left ventricular walls for the cat to be at risk. For many of these cats, including the ones that have come to post-mortem, the amount of thickening of the walls is quite subtle. This means that it may be easy to miss and certainly may have features overlapping with restrictive cardiomyopathy. It is known that in some human families with cardiomyopathy, and in specific mutations, you can have family members with the same mutation and some of them showing classic hypertrophic cardiomyopathy and others showing features more typical of restrictive cardiomyopathy. So we know there is an overlap in these two types of cardiomyopathies in some human mutations for cardiomyopathy. This may help direct as with the NFCs and means that it is possible there may be one mutation responsible for both types of cardiomyopathy that we see in NFCs. That means that if we are looking for cats with early restrictive cardiomyopathy using echocardiographic screening then we are looking for a cat that has a normal left ventricle, and in the early stages will have a normal left atrium; so that means it will look like a normal cat. We have concerns that the conventional methods of screening for NFC may not pick up every affected cat. If this is true, it will be a difficult problem. We will be able to pick up cats with obvious thickening of the left ventricular wall, but cats that have a classic form of restrictive cardiomyopathy may be difficult to identify. This was why we wondered if it may be worth looking at blood tests to identify cats with early restrictive cardiomyopathy. There are new blood tests available, called biomarkers, which may be abnormal potentially (we are hypothesising) even if the echo is normal. This is one of the things we are interested in looking at.

Longer term, we would like to see if there is a specific mutation responsible for cardiomyopathy in NFCs. With any genetic studies, we will need to know quite clearly who is affected and who is normal; otherwise we cannot start looking for genetic differences between those groups of cats.

- **Participant**: Don't biomarkers detect all problems regarding the heart?
- **Virginia**: That's true, the biomarkers we are looking at are not specific for cardiomyopathies but most heart problems that we see in cats are cardiomyopathies. A biomarker is never enough on its own to make decisions, but it can provide useful information.

Slide 18:

Thanks to the very generous contributions from NFC owners and their support for the WINN feline foundation and thanks to WINN for giving us this grant for looking at cardiomyopathy in NFCs. The initial slant of this phase of the study is to try and workout how to exactly identify the type of cardiomyopathy in NFCs. It differs with cardiomyopathies in some other breeds of cat, but has some consistent features. We think all of this suggests it is going to have a genetic basis, but we need to be able to identify who is affected. The first priority is to try and define what NFC cardiomyopathy is. The next will be to test the ability of biomarkers to identify affected cats. The last phase will be to collect blood samples so that in the future we can do DNA analysis to search for a genetic mutation.

- **Participant**: Do you expect to find more than one genetic mutation responsible?
- **Virginia**: It is possible that there is more than one mutation. We feel that where we have a consistent pattern that may be one mutation, but there could be others.
- **Participant**: If you find a mutation, will this substitute scanning or will you have to keep scanning as with Maine Coon breeders.
- **Virginia**: You will have to keep scanning, in human cardiomyopathy there are many mutations. If you want to know about outcome in a particular cat you will have to scan. If you want to know if the cat is likely to pass on the mutation for NFC cardiomyopathy, then just the gene test will be sufficient. If you want to know about the individual cat and whether it will be at risk of developing problems, then you have to keep scanning.

Slide 19: How are we going to define the typical features of NFC cardiomyopathy?

We want to know, does it only affect young cats? What age are we likely to start seeing signs of any abnormalities? Are males affected more than females, as is often found in other types of hypertrophic cardiomyopathy? We want to see if the lack of/low prevalence of murmurs is a consistent feature, or whether we have just coincidentally screened a group of cats that don't happen to have many murmurs; is this going to be one of the key features?

We will want to see if we can identify patterns of wall thickness or echocardiographic features that are typical. Our impression is that we have not seen cats with dramatic hypertrophy, but it may be when we screen more cats that there are some out there. Certainly compared to non-pedigree cats, it seems more common to see dramatic hypertrophy, or it may be that we are just seeing a highly selected referral population. When we look at NFCs in general there may be cats with severe hypertrophy out there but they may be a minority.

The other thing that is difficult to discuss, but really important to our understanding of this condition, is to get information on post-mortem (autopsy). Unless we can look at the hearts from cats that have died from this disease, we won't know some of the really important characteristics. The more cats that we can get a post-mortem examination on, the better our understanding is likely to be of this disease. It's not just seeing the whole heart on post-mortem; we need to do microscopic analysis (histopathology) to identify the arrangement of the heart muscle cells and to look at fibrosis and scarring of areas within the heart. This is going to give us very valuable information.

The other thing that is going to give us useful information is the biomarkers. The two biomarkers that we are interested in are called NT-proBNP and troponin I. NT-proBNP concentrations in the bloodstream will increase as the severity of heart disease increases. Troponin I is released by heart muscle cells when they are damaged, so anything that damages heart muscle cells may result in increased concentrations in the blood stream. That means that we can take a blood sample and measure the level of these biomarkers to get some idea of how much stress the heart is under and whether the heart muscle cells are being damaged. What we don't know is whether these blood tests will be sensitive enough to pick up low levels of abnormalities. One thing we are concerned

about is with young cats that deteriorate rapidly, could these biomarker levels be increasing? Could biomarkers increase before we have changes on an echo scan?

- **Participant**: Those biomarkers have not been clear in other cats, why would it be different in NFCs?
- **Virginia**: If we are looking at a form of restrictive cardiomyopathy, we believe it is more likely to be abnormal than the echo. I think we are still going to see cats that have normal findings on echo and cats that have mild thickening of the left ventricular heart walls on echo where we will have normal biomarkers. Having mild changes on a scan and normal biomarker levels would be reassuring that the cat is not going to develop serious problems. There have been a couple of studies looking at Maine Coon cats and NT-proBNP didn't look a very sensitive test in that particular study, but I think it all depends which questions we are asking. Our concerns are that we could have cats with quite serious problems that we are not able to pick up just on echocardiography alone.

Our intention is to collect blood samples from cats that have been screened with echocardiography and we will just look at the results and see if they seem to give the same information, or whether they give different information. If we find that the biomarker results are the same in all cats, then that will tell us that this is not going to be a fruitful path to go down. If we find that some cats have abnormal biomarker results, despite a normal scan, then these will be cats that we would want to keep a very close eye on.

Slide 20:

For each cat that is screened, where we know what the heart looked like on a scan or biomarkers, we will want to store red blood cells from the blood sample that we have taken so that in the future we can go on to DNA analysis. We are not going to be able to undertake genetic studies until we know which cats are definitely affected and those cats which are highly likely to be normal.

- **Participant**: In a cat with equivocal RCM, NTpro-BNP has come back normal.
- **Virginia:** I think it would be a big assumption to make that because in previous studies NTpro-BNP hasn't been abnormal in very many cats that the same is going to be true in NFCs. Troponin I has not been looked at very closely and I think that until we look we don't know the answer, so it is too early to say that this test is not going to be useful.

What we need for genetic studies is DNA from cats that are not just normal on echocardiography and biomarkers, but cats that are normal and older. We don't know the age of onset of cardiomyopathy in NFCs, so the older the cat is and is still normal the more confident we can be that that cat is genetically normal. For cats that are affected, the age doesn't matter. The ones that we know will definitely be affected are the cats that have died of heart failure and have cardiomyopathy confirmed on post mortem. What do I mean by older? The older the better and ideally I would say 7 years or older. There has been very little work done on the age of onset of hypertrophy or characteristic changes. We know that some NFCs will be affected by the age of 2 years, what we don't know is whether cats that have milder forms of cardiomyopathy may only start to get changes at 3, 4 or 5 years of age. The more we screen, particularly if we are able to do serial studies, where

we examine the same cats over time, that will give us more information about how old a cat needs to be and look normal before we can say they are normal. In the mean-time if you have normal 12 year old cats, I think we can be pretty confident that those cats are genuinely normal.

Slide 21: What have we done so far?

We started with the WINN project in March and have screened 132 cats and done 138 scans. Here is a graph of the breakdown of age; you can see that the majority of cats that we have screened have been young cats. This is understandable, people are anxious that they may have a young cat that they are concerned may be severely affected. We have scanned some cats less than one year of age, and again if you have a young cat related to affected cats we know there have been a small number of cats affected before 1 year of age. Normally when screening in other breeds, we would say to wait until a year of age but this seems unfair when cats can be severely affected before 12 months. We have also had some old cats, 5 cats have been 11 years or older and we are particularly interested in seeing older cats.

- **Participant**: Do you need to do all the scans yourself or can we go to other Pawpeds listed cardiologists to have cats screened elsewhere?
- **Virginia**: I think in some ways it is very helpful for us to see the whole scan, rather than just have the numbers measured by someone else, but I think more information is good information. Even if you have scans done elsewhere, if we can gather together that information (we have been using the Pawpeds database looking at patterns of inheritance) that is also useful. You don't have to come to the UK to be scanned here. You can use Pawpeds listed or boarded (ECVIM or ACVIM) cardiologist elsewhere. Pawpeds listed cardiologists are not listed in every country, but if you have an ACVIM or ECVIM boarded cardiologist, that is also extremely useful data for us.
- **Participant**: Is it possible to send the whole scan together with the measurements and blood samples?
- **Virginia**: We need to sort out the protocol for sending blood samples; there are some quite strict regulations about sending blood samples. We will be working on giving further details, not during this webinar, but subsequently on how we can transport blood samples. In the mean-time if the sample is taken and stored locally that will be helpful.
- **Participant**: Would it be possible for you to go into other countries to try and do further screening clinics?
- **Virginia**: We haven't made plans to do that and right now one of the limiting factors is my availability, I have very limited time. However, we have two cardiology residents working here who I hope will be listed on the Pawpeds database in the near future – Kieran Borgeat and Rosie Payne. Hopefully the more of us doing it within this particular research group, the more cats we will be able to get screened. I wouldn't rule it out just because we don't have plans at the moment.

Slide 22:

If you look at the breakdowns of which cats are affected and which are not, you can see it's encouraging that the majority have been normal when we use a definition of less than 5mm wall thickness. We have a proportion of cats that are affected and a smaller proportion of equivocal cats, where we can't call them affected but we can't call them normal either. There is a minority with more classical restrictive cardiomyopathy with left atrial enlargement. We have just completed a study looking at over 780 non-pedigree cats in rehoming centres/shelters conducted by Rosie Payne, one of our residents. The prevalence of cardiomyopathy in non-pedigree cats is about 15%, those are mostly very mild and we don't expect a high proportion of those cats to go on and develop problems. We are going to follow those cats over the next 10 years so we get some idea of the usual rate of progression in cats that are diagnosed in cats with HCM. The same may be true in NFCs, just because they are diagnosed with HCM we don't know that they are going to go on and die of HCM. We suspect many of these cats will remain free of symptoms and may never develop problems. One of the things we are interested in looking at is what happens to cats that we screen and are we looking at two populations? Do we have a group of cats that are very young and progress very rapidly and another group of cats that have a more classic form of mild HCM that remain symptom free for many years; like my Bengal?

Slide 23:

We have also been looking at post mortem data on some cats. This is one of the reasons why a definitive post mortem is **extremely** important. We've had 4 cats that were classified as having hypertrophic cardiomyopathy on post-mortem. Two had a form which had some features of HCM and some features of RCM. We had one cat which looked more typical of arrhythmogenic right ventricular cardiomyopathy (ARVC); that was a surprise to us! One cat was diagnosed with myocarditis and one had a congenital heart defect. We can't assume that every cat which dies suddenly or has heart failure has HCM. We may be dealing with something that can present in multiple different ways.

Slide 24:

Looking at the family tree, these cats (cats that we have post-mortem results for) are all related in some way, some more distantly related than others. The more data we get the better it is for working out patterns and working out what is going on.

- **Participant**: Could the two populations, HCM and HCM/RCM, be related to genetic status?
- **Virginia:** We don't know that there are two populations. We don't know if we are dealing with two different HCM mutations or whether we have one mutation with different modifying factors. In human families with one mutation, although they share a mutation some family members will have a severe form and some will have a mild form. It doesn't

necessarily mean that we are not dealing with the same mutation. In humans it is believed that there are other genes which may influence the expression of the mutation gene. In humans you can have family members which might have a specific HCM mutation, and it may be other genes that are influencing whether they have a severe or mild form. I don't think we can make the assumption that just because there is a difference in the rate of progression that we aren't dealing with the same mutation – it could still be one mutation.

Slide 25:

What we really need more than anything is having more post-mortem data. The more post-mortem information we can get, particularly on those that are severely affected, the more we will know about what is going on. If you have a cat which dies outside of the UK it is difficult for us to get information on that cat, but we will be working on what to do. It may be that we can identify pathologists in your country that will enable us to get the post-mortem data. It is really important, not just to rule out other conditions and to confirm that we are dealing with cardiomyopathy, but also because it will tell us more about the specific features. The thing that is very convincing with post-mortem data is that we know for sure those cats have the serious form of cardiomyopathy. There may be other cats that have mild increased ventricular wall thickness where it's difficult to know what their outlook is. On post-mortem we know we are dealing with the ones that have the 'bad form' of cardiomyopathy. We are particularly interested in screening cats related to those who have died of their heart disease and particularly older cats. We are also interested in screening cats in families that don't seem to be affected by cardiomyopathy. If we have families where no cat is known to have developed problems, we are interested in looking at those as well to form a comparison for cats in the other families.

- **Participant**: We have a pathologist working in DK with Pawpeds listed vets on HCM. Are you going to try and identify pathologists yourself, or do you want breeders to contact the Pawpeds vet they work with and ask them to contact you?
- **Virginia:** Either way we would be interested in information from other pathologists and would be interested in information from the vets that they work with – any information at all! I hope that none of you has to deal with a cat that dies of cardiomyopathy, but if that happens we would definitely like to hear about it and any information that comes from that will be very helpful to us.

Slide 26:

We will need to sample as many cats as possible, just having a blood sample is not enough – we need to know what the heart was like. We would like blood from any cat that has been screened, but ideally screened as late as possible. Having a blood sample from a 1 year old cat is not particularly useful unless that cat develops problems in the future, or if we have another scan when the cat is over 7 and is still normal. We don't necessarily need to keep obtaining repeated samples if we are just looking at retaining the DNA of that cat. If we are looking at biomarkers, then we want

samples at the same time as the cat was scanned. Also we really need to know what happens to these cats, so if we have screened a cat we need to know what happens when they get older. If any cat develops cardiomyopathy later on, signs of congestive heart failure, sudden death, or a clot (arterial thromboembolism; ATE) we really need to know about that. If your cat lives until 16 and ends up dying of something else, we would like to hear that too! The more outcome data we have, the better we will be able to interpret our DNA samples. We will not just use echo results; we want to make sure that we include the information on young cats that die prematurely, biomarker results and post-mortem results to decide whether a cat has cardiomyopathy. Only when we have a set of DNA samples from cats which we are confident are normal and a set from cats we are confident are affected, and ideally as unrelated as possible, will we be in a position for identifying a mutation.

- **Participant**: I am going to try and engage Utrecht University for your project.
- **Virginia**: Thank you for considering contacting Utrecht. We know the cardiologists there, so we will welcome as much collaboration as possible.

Slide 27: Why would it be helpful to identify a mutation?

Once you know which mutation, or which change in a gene you are looking for you don't need very much DNA and can look for it using mouth swabs; as is possible for Maine Coons and Ragdolls. It means you can then make more informed decisions about breeding. If you have a cat that is carrying the mutation then you can decide in a more informed way what you do. It will allow you to monitor particularly closely those cats with a responsible mutation. It is going to take a while; you see that we have some specific problems in NFC with identifying who is affected. Until we solve those problems we can't even start looking for possible mutations in our DNA samples. I know Kate Meurs has started looking for possible mutations in the 8 main genes that usually cause HCM in humans and in the 3 NFCs included she didn't identify any changes; but that was a 'quick look to see' and there may be many more subtle differences which won't necessarily have been picked up on that particular study. So, it's going to be a while I'm afraid.

- **Participant**: Could the difference in severity or speed of progression in cardiomyopathy signs be related to whether they are homozygous or heterozygous for the mutation?
- **Virginia:** It is possible that having two copies of the abnormal mutation (homozygous) would give you a worse prognosis than just having one (heterozygous). We have just started looking at this in our Ragdolls; our resident Kieran Borgeat has been looking at survival in Ragdolls according to whether they have two or one copy of the mutation. Survival time seems to be shorter in Ragdolls who are homozygous (two copies of the mutation). This may explain why we see some older NFCs with mild hypertrophy and severe disease in young cats. Something that has been seen in humans with HCM is that people with a known HCM mutation in common with other family members, have a much worse disease than the other family members if they also have another HCM mutation (more than one). There are many HCM mutations that have never been identified in humans and currently there are over 1400 different mutations in people that can cause HCM. We have only identified 2 mutations (in Maine Coons and Ragdolls), so we are very behind compared to human HCM. It may be that we are talking about homozygous versus heterozygous or we could be looking

at cats with more than one versus just one mutation, which may be explanations for the difference in severity we observe.

Slide 28: Can the measurements of wall thickness on echo vary over time?

Absolutely there can be a variation in wall thickness. The wall thickness throughout the wall is not even, there may be focal areas of thickening and other areas where the wall thickness is more normal. Whether you get the same measurement on two studies will depend on whether somebody has measured in the same place or not. There may also be differences between m-mode and 2D. This graph is from a study we did a few years ago where we compared the values on 2D and on m-mode and found that a measurement on one method may not correspond exactly with a measurement on the other method.

There can be some fluctuation in wall thickness itself. If we start with a classic form of HCM with thick walls of the left heart, we are starting to realise how little we know about what happens over time in affected cats. What we expect to happen over time is that as the cat copes less well, we may still have the same wall thickness but the left atrium becomes enlarged as the pressure starts to go up in this chamber. At that stage we start to worry that the cat may be at risk of developing congestive heart failure or thromboembolism. What we are also seeing in these cats that have a big left atrium is that sometimes the wall thickness starts to vary and instead of being even throughout the heart, there may be some areas where it is thinner than others. This may be related to death of areas of heart muscle cells. Some cats may lose some heart muscle cells as the disease becomes more advanced. In some cats, we will see whole chunks of ventricular wall just die. This may be associated with myocardial infarction where you have really severe disease and the wall of the ventricle becomes replaced by a scar; which result in a difference in thickness of the walls. So, it is possible for wall thickness to go in either direction. You can get cats whose walls become thicker as the severity of the disease gets worse and you can also have cats whose walls get thinner as the disease gets worse.

- **Participant:** Is this type more often seen in mild, moderate or severe cases, or does it not matter which?
- **Virginia:** We definitely see big changes in cats with very severe disease. There is a lot of difficulty in knowing where HCM ends and RCM begins and we don't even know if they are definitely different diseases or the same disease at different stages. Some people think that you could start off with a cardiomyopathy that looks like HCM, with thick walls and a big left atrium, and then lose some of the muscle through cell death to end up with a heart that looks like restrictive cardiomyopathy.
- **Participant:** Would this loss of wall thickness still be recognised by a cardiologist as abnormal?

- **Virginia:** I think by the time you have a big left atrium, then yes it would certainly be recognised as abnormal. What we don't know is whether restrictive cardiomyopathy represents a very advanced stage of HCM or whether it's a form of cardiomyopathy where the walls just don't get very thick even though the heart muscle cells are abnormal. This is still being debated and we will need more post-mortem data to be able to answer these questions.

Slide 29:

Here is an example; this is not a Norwegian Forest cat. On the left, this is a short axis view; a transverse section of the left ventricle (arrow demonstrates different structures including papillary muscles). This cat has very big papillary muscles and there is some increase in thickness of the walls of the left ventricle. We were quite confident this cat had HCM, she was a non-pedigree cat.

On the right is the same cat, in the same view, 4 years later. Now she doesn't look like she has HCM. She has maybe slight thickening (points), but the wall of the left ventricle in this area (points) is tiny, it's very thin. We just have a scar and a very thin looking papillary muscle. This cat has had death of part of the left ventricle and has gone from looking like a cat with HCM to looking like a cat that may have RCM or what we sometimes call end-stage HCM. Almost certainly she had a myocardial infarction that was responsible for this.

- **Participant:** Can't there be a variation in numbers in a totally healthy cat?
- **Virginia:** Most often what's happening when we get slight variation is that we are measuring just slightly different areas of the same ventricle. This is very easy to do and does not mean that the person doing the scan is not doing a good scan; it may be that they are just slicing through a slightly different part of the ventricle. It doesn't necessarily mean that there is anything wrong. To recap, you can have fluctuation in the numbers partly due to sampling a different bit of the left ventricle or it can be due to the change in actual thickness of the heart; which is more common in severe cases. In a healthy cat, you would expect some variation and change in numbers even with highly skilled cardiologists.

Slide 30: Are only the measurements used to decide if a cat is affected?

The measurements are part of making that decision, but we can look at other criteria. The papillary muscles are also something that we look at. Here in short axis view of the left ventricle, you can see very long, tall looking papillary muscles which doesn't look like a normal cat at all. If a cat has normal wall thickness everywhere else that we are measuring, but the papillary muscles are very big then we would call this cat equivocal.

This cat has thick walls throughout and during contraction the papillary muscles are filling the entire cavity of the left ventricle, that's called end-systolic cavity obliteration and would make us suspicious that this cat was affected even if we didn't measure extremely thick walls.

- **Participant**: All the examples you have shown have an enlarged left atrium. Can this feature be used to identify cats with cardiomyopathy?
- **Virginia**: A big left atrium is a powerful indicator of something being abnormal, so this is a key significant finding and suggests the cat may be at risk.
- **Participant**: If you have a cat with increased wall thickness that then goes away, do you still call it HCM?
- **Virginia**: We would want to look at multiple different factors. If we are using a strict cut-off point and it goes from being just a tiny bit thick and then down again, I think it probably isn't too much of a worry. If you have a cat that's clearly thick and has a big left atrium, then has goes back to normal thickness still with a big left atrium then I would be concerned about that cat.
- **Participant**: How often do you expect problems with valves in HCM cases?
- **Virginia**: It is easier to tell on histopathology than ultrasound. I was in a meeting in Minneapolis last weekend on human HCM, it was nearly 3 days of discussion with top human HCM experts and they don't even know if problems with valves are a primary or secondary problem in HCM. There are more questions than there are answers. In human HCM they will see the longer mitral valve leaflets, but this happens in people who have no mutations. I think we are still at the data gathering stage.

Slide 31: What testing protocol should be used for breeding cats?

I think it makes good sense to have cats screened with echocardiography to check if cats are affected. We can't tell on physical examination. What we don't know is how early we expect to see abnormalities. It makes sense to screen cats over 1 year of age, but if there is a known history of cardiac death you may want to screen earlier than that. Nine months of age may not be too early to screen a cat that is related to a cat that has dies of cardiomyopathy. We will only know if it is cardiomyopathy if you have a post-mortem done of if there has been an echocardiogram that confirms we are dealing with cardiomyopathy. Don't forget that just because you have a normal echo at one year of age doesn't mean that a cat can't develop cardiomyopathy later on. I'm afraid if you want to know if a particular cat is affected that means you need to continue scanning. We don't know the age at which HCM develops, so we don't know at what age it is safe to call a cat definitely normal. We think that 7 years is a reasonable age, but we don't know. Screen from one year of age and ideally continue screening.

Slide 32: Is aortic stenosis a problem and is it inherited in NFCs?

One of the problems is that it can be difficult to differentiate aortic stenosis from HCM with dynamic outflow tract obstruction. Here we have a Burmese cat, where the walls look quite thick (left ventricular hypertrophy). This is the aortic valve here (points) and there is an area of narrowing just at the exit from the left ventricle and entrance to the aorta. There is also an area of tissue causing obstruction to blood flow ejection.

That's something that in dogs we would call aortic stenosis, but in cats that start off with dynamic obstruction, because of abnormal movement of the mitral valve, that can create scar tissue in this area. So, it can end up difficult to differentiate from aortic stenosis, which is a congenital problem where they are born with fixed narrowing at the exit from the heart, from cats with HCM and obstruction due to abnormal valve movement that then develops scar tissue. Aortic stenosis is rare in cats, so I think a cat with aortic stenosis we would be suspicious that it could be another variation of HCM. There are many, many variations on HCM and I think that we should consider that all cardiomyopathies are a variation on that until proven otherwise.

Slide 33: Any advice on biomarkers?

It is difficult to give any advice at this stage. All of the work that has been done on biomarkers suggests that it is most useful for identifying cats at high risk of developing clinical signs. If you want to identify cats with a risk of cardiac death in the near future, this can be a good way to look. In NFCs, we don't know how the test will perform. We are going to run a lot of the tests at one time, rather than just a few here and there; so we don't have any data to show at the moment. In non-pedigree cats, we have been identifying an increase in wall thickness and in cats with an NT-proBNP level over 50pmol/l; so this level should be a trigger to get the cat scanned. With Troponin I (hsTnI), if the blood test comes back as more than 0.2 ng/ml; that could indicate an abnormality and that cat should be scanned. Troponin I is the biomarker for damage to heart muscle cells, so that may be particularly abnormal in cats that are due for having abnormal clinical signs. So, in either of those situations you should consider getting the cat scanned.

Slide 34: Should you scan before first breeding?

Ideally yes, you can't tell on physical exam so there is no way of knowing whether a cat is affected. Although many cats may be normal at a young age, there may be more severely affected cats that will not be normal at that age. We want to know if we can pick up affected cats at that age using biomarkers, which is a much more practical test for people to use. Right now there is nothing to beat an echo done by a cardiologist.

Slide 35: Is it possible to overestimate wall thickness on echo?

Let's look at M-mode versus 2D. Here's an M-mode view with a short axis like I showed you earlier, a cursor is placed across the middle of the left ventricle and then the points of the heart crossed by that line are plotted against time. Here we have an ECG; this is when the heart contracts and this is when the heart relaxes. What we are interested in for measuring wall thickness is how thick the wall is when it's at its thinnest, which is when the heart is completely relaxed. In this cat, we have nice clear lines and it looks quite straight-forward to calculate where the wall starts and where the wall ends. So, this looks like an easy M-mode to measure.

They are not all that easy though. Here's a couple of 2D images and here we have the short axis view, the same view as we use for M-mode, and usually measure in multiple different places. Here's a long axis view and again we are measuring the wall in multiple different places, looking for where it might be thickest.

End-diastole means we are measuring when the hart is relaxed and the wall is at its thinnest. If you don't measure at that time point, you get an artificially thick measurement. The other question which could influence the result is whether you include that white line that's the lining of the heart (endocardial border). In some NFCs that seems to be quite bright and quite thick. It is not known whether that is a normal finding in NFCs, or whether it's an abnormality related to RCM - we just don't have that information at the moment. It could make a difference to wall thickness measurements if those endocardial borders are included.

The other thing that could make a difference is that if you look at the inside of a heart there are tiny strands of tissue called false tendons (points to white line). If you think this is part of the lining of the heart and include it in your measurement, then instead of measuring the actual wall thickness it would be possible to include that as part of the lining of the wall and therefore overestimate the wall thickness. It is important that you exclude false tendons in your wall measurements, which I think is more difficult to do in M-mode than it is on 2D, because it can potentially result in an artificially thick measurement.

Slide 36: Why does the Maine Coon test not work in NFCs if they share common ancestors?

We know in human HCM there are 1400 different mutations, so it is not a surprise if Maine Coons have one mutation and NFCs don't seem to have that same mutation. It may be there is more than one mutation in Maine Coon cats.

Slide 37: Is dehydration important in testing?

We know in severe dehydration that can reduce the volume of circulating blood, make the diameter of the left ventricle smaller and can make the walls thicker. It probably has to be severe dehydration to cause that, not just the cat not having a chance to drink since breakfast or the night before the scan. If cats are behaving normally and have not been unwell at home, they are probably not dehydrated enough to have any effect on wall thickness. It is important in cats that are ill with something else that if they are dehydrated it is not a good time to do an echo to see if their heart walls are too thick. Some of these cats might have a murmur, but you need to wait until they are over the illness before you measure their wall thickness on echo.

Slide 38: Should the testing guidelines be different for different weights of cat and different breeds?

We are starting to see differences between different breeds and there may be some influence of weight. We looked at the shape of the heart in NFCs compared to non-pedigree cats and looking at the ratio of the diameter of the ventricle to the thickness of the walls, it seems NFCs have relatively thinner walls compared to most non-pedigree cats. It's possible that we may be looking for more subtle increases in wall thickness in NFCs then we might look for in a non-pedigree cat. This would fit with Jens Haagstrom's suggestion that we should have the cut-off of at least 5.5mm rather than 6mm, which is a more common number that we use in non-pedigree cats. It may be that the normal wall thickness in NFCs and Maine Coons is a little bit smaller.

We have looked at the influence of body weight on wall thickness. The line on the graph shows the 5.5mm cut off value used to separate normal from abnormal. You can see that in normal cats there is a slight increase in wall thickness as the weight of the cat increases, but it is a pretty subtle difference. Being a bigger cat shouldn't necessarily be enough to push you over that cut-off, but we will continue to gather information from cats we have scanned and the Pawpeds database.

Slide 39: Is HCM in older cats different from in younger cats?

We think that the form we see in young cats is a particularly aggressive form. However, we see older cats with milder changes and with irregular distribution of hypertrophy. It may be that some of these more variable types of HCM or end-stage HCM in older cats are because they have more time for death of heart muscle cells and scarring so that they look different. We are currently looking at a population of geriatric cats over 9 years of age that are very well characterised to see if we can identify features of normal older cats, then we will have a better idea of how to interpret findings in older NFCs.

Slide 40: How much HCM is 'secondary'?

A number of different things can affect wall thickness and we need to take these into consideration. Rather than talking about HCM, we can talk about an 'HCM phenotype'. A phenotype is what we actually see and a genotype is what genes are present and what mutations are present. All we have to go on to work out what genotype may be present is what the heart actually looks like. So, we recognise an HCM phenotype as a left ventricle that has thick walls. Other things that can cause thick walls of the left ventricle include: **hypertension** (high blood pressure), **hyperthyroidism** (overactive thyroid gland) and **acromegaly** (hypersomatotropism; an excess production of growth hormone). Tumour infiltration can also end up affecting the left ventricle, occasionally lymphoma can infiltrate the heart but post-mortem information and histopathology will tell us about that. It is pretty easy to rule out these conditions, as we can test specifically for them, and if they are not present we usually assume that it's HCM that we are dealing with. If we have one of these conditions it's difficult to know whether the increased left ventricular wall thickness is due to one of these conditions or

whether it had HCM first and got even thicker walls when the cat developed hypertension, hyperthyroidism or acromegaly. Strictly speaking, we can't say if a cat has HCM or not if it has severe hypertension (very high blood pressure), because it could be due to the blood pressure.

There is some overlap also with RCM phenotypes and end-stage HCM. Classic HCM can end up looking like end-stage HCM if the heart no longer contracts properly. If we get so much death of the left ventricular wall that the wall thickness ends up normal, it could look like a restrictive cardiomyopathy phenotype. It can be difficult if you just scan a cat for the first time to work out whether it started off with true HCM, or developed increased wall thickness because it became hypertensive or whether it had both things going on. Likewise if you have a cat with normal wall thickness and a big left atrium, did it start off with HCM and then develop a restrictive phenotype?

- **Participant**: Can the kidney itself cause an HCM phenotype?
- **Virginia**: We believe the main way that kidney disease could cause increased wall thickness is if it was associated with hypertension. If hypertension was present that could increase wall thickness.
- **Participant**: Have you ever seen secondary HCM in Addison's disease?
- **Virginia**: Addison's is rare in cats, but could cause similar effects to dehydration and increased wall thickness on a scan. The wall thickness should go back to normal once the cat is no longer dehydrated. With a stable Addison's patient you would expect normal wall thickness.
- **Participant**: What about excessive aldosterone secretion?
- **Virginia**: Again, it probably wouldn't be sufficient to cause an HCM phenotype.
- **Participant**: The heart is known to express receptors for aldosterone.
- **Virginia**: In chronic kidney disease in other species we don't see increased wall thickness. In some experimental models with left ventricular hypertrophy, they were responsive to blocking the aldosterone receptor. So far we have not seen a big response and regression of hypertrophy with blocking the aldosterone receptor (aldosterone antagonists). We are currently conducting a study looking at geriatric cats, looking at cats with chronic kidney disease but are not hypertensive and comparing them with aged matched cats that do not have chronic kidney disease or hypertension to see if there is a difference in wall thickness. Right now we don't think that chronic kidney disease alone can cause an HCM phenotype but have designed a study specifically to try and answer that question by comparing cats of the same age that do and do not have chronic kidney disease and where cats in neither group have hypertension. We may even have information on aldosterone secretion on these cats, they are part of Jonathan Elliott and Hattie Syme's group of geriatric cats and they have also been doing work in this area. So, although I can't answer your question absolutely now, we are trying to look at that very question.

Slide41:

For any cat with an increased left ventricular wall thickness, you should always check that blood pressure is normal.

Slide 42:

You should always check blood thyroxine levels in older cats.

Slide 43:

If a cat is diabetic, then you should consider acromegaly (hypersomatotropism).

Slide 44: Thank You

Many thanks to many of you:

- ***Yve Hamilton-Bruce*** for getting me started off on this
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- Above all, the Norwegian Forest Cat ***owners and breeders*** who have contributed to our study so far, whether via contribution to the ***WINN feline foundation*** or bringing in cats or submitting cats for post-mortem – we are very, very grateful!

RVC Royal Veterinary College University of London

Live question and answer session

- **Participant**: It is frustrating that not all countries use the same cut-off values, such that cats could be scanned normal in France and called mild HCM in Scandinavia. What can be done to use the same values?
- **Virginia**: I agree entirely that it is very confusing to have different cut-off values being used in different places. There is no magic number, there is no number above which you can say all cats are abnormal and below it all are normal. There is an arbitrary number used in humans, so they say that if you have a wall thickness above 15mm you have HCM and below that it's likely that you don't have HCM; but there will always be a big grey area. When we are looking at the general cat population of non-pedigree cats, our impression is that wall thickness is much thicker in normal non-pedigree cats than it is in normal NFCs. I believe that NFCs should have a different set of normal values to non-pedigree cats. The lower value for the normal cut-off point, that is generally used with Pawpeds and Scandinavia where many of the screened cats are either Maine Coons or NFCs, I think the lower cut off may be appropriate. Most of the values on Pawpeds have been derived from M-mode and the wall thickness tend to be a little lower on M-mode than it is on 2D because you only sample in two places of the walls, whereas on 2D you can pick the thickest area. I think the suggestion that normal values are a wall thickness under 5mm is likely to be a normal cat. I think everybody agrees that 6mm or more is likely to be abnormal. We are talking about whether we use 5.0, 5.5 or 6.0mm as the cut-off point and I think the grey zone is somewhere in that region. I think that for NFCs and Maine Coons that figure is going to be lower than for non-pedigree cats but is also complicated by body weight. I think we are being unrealistic if we think it is going to be as simple as saying under and over a certain value. I know that's frustrating but we are using an insensitive technique for making the decision, which is why we are particularly interested in post-mortems of cats that have died of HCM because we know those cats are abnormal and definitely affected. Mild increases into the grey zone or 'equivocal' values we don't know completely how that translates into a possible genetic problem. I think if your cat has just edged over a strict cut-off point, you need to take lots of other factors into consideration before you make big decisions about removing that cat from the breeding population, for example. What I would like to see is a consensus of group agreement on exactly how you do the measurements, because if people are not measuring in the same way then that in itself can be enough to account for fractions of a mm. That's all we are talking about, 1mm difference between the strictest cut-off and most generous cut offs for normal. I would like guidelines for how we should be measuring and acquiring images.
- **Participant**: If a cat has a 1.5mm increase in left ventricular wall thickness, is that still normal if it is below the cut-off or is the increase in itself a cause for concern?
- **Virginia**: I would say that if it's below the cut-off then I wouldn't be concerned.
- **Participant**: So NFC values are likely to be lower than normal non-pedigree cats or Maine Coons?

- **Virginia**: I think they are lower than non-pedigree cats and are likely to be similar to Maine Coons.
- **Participant**: I have seen scans for Maine Coons in the USA where values of 6.0mm and above where considered normal based on the fact the cat was over 7kgs in weight.
- **Virginia**: This is the difficulty, there is never going to be one single value fits all. We don't rely only on the values, so if you have dynamic outflow tract obstruction, abnormal mitral valve movement or very big papillary muscles, that would make us more likely to suspect a cat is affected. The effect of weight on wall thickness is minor compared to the effect of having HCM.
- **Participant**: Do NFCs have lower values or less variation?
- **Virginia**: I think that NFCs have less variation than the non-pedigree cat population; again we are doing more work to look at this.
- **Participant**: If we increase cut-off values to 6mm, we have not many cats affected with HCM.
- **Virginia**: I agree that if you increase the cut-off values then you will have fewer HCM cats, but we have seen cats on post-mortem that have typical features of HCM on histopathology who had wall thickness of under 6mm when we canned them. If we increase the cut-off to 6.0mm I believe we will start missing cases of HCM in NFCs. Again, this is why ideally we would have more information on cats that are scanned with echocardiography and then we get post-mortem information on the same cat. This is the only way we are going to get answers to these questions. Looking at non-pedigree cats in rehoming centres, we found that it seems to be a separate population of cats with thicker walls; it's not a continuous distribution of wall thickness. It looks like there is a natural difference if you put the cut-off value at 5.7mm so I believe the true cut-off is somewhere between 5.5 and 6.0 mm and that is a tiny difference. Most normal cats should be well away from that distinction and probably should be less than 5.0mm.
- **Participant**: There is research in rats where they have looked at hormonal influence on salt channels in kidneys and high blood pressure in relation to developing HCM. Could there be a hormonal influence in HCM?
- **Virginia**: We think there could be factors that influence development in HCM. In humans with HCM you can have different individuals with the same HCM mutation and if you have a separate hormonal influence in addition this can make a difference as to whether you have severe or mild hypertrophy. This is very early days even in human HCM and rats are not a very good model of HCM. There is not a very good naturally occurring model of HCM; cats are the most similar to human HCM of all the animals.
- **Participant**: The population of older NFC in the UK is quite small with respect to its variation in lines. Could this be creating a bias in your research population?
- **Virginia**: This may create a bias in the cats that we are seeing, but we would welcome information on older cats in other countries.
- **Participant**: How thin are walls that are too thin?
- **Virginia**: Normal cats may have walls just above 3mm, in the 2mm zone is probably below normal wall thickness.
- **Participant**: Do you prefer 2D to M-mode?

- **Virginia**: Yes, I prefer 2D scanning because I feel I can see what I am looking at. You need a high quality machine with a high enough frame-rate that gets you to the end of diastole; you need the end-diastolic frame. If you have a lower specification machine, M-mode is more likely to get you the end-diastolic frame where the wall is thinnest. We do both so that we can make comparisons with other peoples' normal values. It would be good to get everyone doing things identically and we are working on that.
- **Participant**: Some Finnish breeders have gotten the impression that scanning cats over 6-7 years old for genetic HCM is useless due to a high likelihood of secondary HCM from other reasons. Is there any evidence to support this?
- **Virginia**: In older cats with thick walls, if you can rule out hyperthyroidism, hypertension and acromegaly then you should assume HCM. Increasingly in human HCM they are finding people even up to their 90s with HCM. So, we should not rule out HCM just because a cat is old.
- **Participant**: Is your new scanner giving different results to the old scanner?
- **Virginia**: I sincerely hope not! These machines are tightly calibrated and the results should not be different.
- **Participant**: How do we know if the equipment our vet has is good enough?
- **Virginia**: I know many extremely good cardiologists with good machines that still prefer M-mode. I was talking to Phil Fox at the weekend, who has done more in HCM than anybody else and published one of the first papers in the human cardiology journals on HCM, and he still thinks M-mode is best; I am working on convincing him. Meanwhile, watch this space – we do both 2D and M-mode because we want to be able to make comparisons. If your vet is an approved screener, it is very likely that they have good enough equipment. Unless you are using very old, cheap veterinary equipment frame rates are very fast and I think a bigger factor is the person doing the scanning. I think results may not be reliable if you are having your own primary care vet doing the scanning. I think one of the most difficult areas in cardiology is to screen healthy cats for HCM; it's very, very challenging. Unless you have the training and experience it is not a job for general practice vets. Pawpeds equipment should be advanced enough; they use Vivid 7 which is what we use.
- **Participant**: What about a diagnosis based on only one measurement for each value to check?
- **Virginia**: I think we usually like to make multiple measurements even if only one value is entered on the form. We like to look at lots of different areas of the heart, which is why we like to look at 2D. For cats that are severely affected, their M-mode is going to be abnormal as well but you should make multiple measurements.